Biomaterials-Based Regenerative Therapies for Intervertebral Disc

Subjects: Surgery | Medicine, Research & Experimental
Contributor: Hideki Sudo

Intervertebral disc (IVD) degeneration is a common cause of low back pain and most spinal disorders. Recent advances in regenerative medicine and tissue engineering suggest the potential of biomaterial-based IVD regeneration therapies. These treatments may be indicated for patients with IVDs in the intermediate degenerative stage, a point where the number of viable cells decreases, and the structural integrity of the disc begins to collapse.

Keywords: Biomaterials ; nucleus pulposus ; Intervertebral disc ; Regeneration

1. Soft Biomaterials for NP Repair and/or Regeneration

Ideally, biomaterials for nucleus pulposus (NP) repair should accommodate both the biological and mechanical aspects of Intervertebral disc (IVD) repair and regeneration [1]. The objectives required for a soft biomaterial-based NP repair approach in terms of the biological response include the soft biomaterial

- being biocompatible, non-toxic, and safe in vivo;
- support cell survival;
- promote ECM formation;
- reduce inflammation; and
- inhibit pathological fibrosis [2].

In terms of biomechanics, the soft biomaterial should (1) remain within the disc under in vivo loading conditions and (2) improve biomechanical disc function and spinal stability.

The concept of using biomaterials that can be injected into IVDs began in the 1960s when Nachemson et al. [3] proposed a method of injecting vulcanized silicone into degenerated discs for nucleus augmentation. Over the subsequent decades, IVD substitutes have been developed to restore disc function [4]. NP replacement using injectable, in situ curable materials can maintain immediate disc height and mechanical disc weight-bearing capacity [5][6][7][8][9] but is restricted by the risk of complications, such as implant dislocation and endplate damage, and by the limited potential for biological repair [5][6].

2. Biological NP Repair and/or Regeneration Using Soft Biomaterials

Many polymeric materials have been experimentally investigated for use as NP-regenerative soft biomaterials. Biomaterials are hydrogels or solid scaffolds and can be divided into synthetic biopolymers and natural biomaterials [10]. Synthetic materials include poly (D,L-lactide) (PLA) and its derivatives, polyethylene glycol (PEG), polycarbonate urethane (PU), and poly (ε-caprolactone) (PCL), some of which can function as both hydrogels and solid scaffolds [11][12][10][13][14][15][16][17][18]. Synthetic hydrogels consist of polymer networks that can absorb a large amount of water, are easy to modify, and can be consistently and highly tunable [10]. However, most production processes of synthetic hydrogels involve the use of reactive reagents and require the complete removal of contaminants and unreacted reagents [4][19]. In comparison, natural polymer-based biomaterials mainly include hydrogels, such as alginate, agarose, fibrin, hyaluronic, collagen, chitosan, and carboxymethylcellulose [20][21][22][23][24][25][26][27][28][29][30][31][32][33][34][5][10][13][35][36][37][38][39][40][41][42][43][44][45][46]. These natural hydrogels have been extensively studied for NP tissue engineering due to their excellent biocompatibility and biological activity and their participation in the physiological turnover process [5][13][47]. A number of in vitro studies have shown that these hydrogels support cell survival and induce differentiation of residual NP disc cells and stem cells [36][38][40][41][42][45][48].

To achieve intrinsic and sustainable disc regeneration, soft biomaterials are required to support cell survival and induce in vivo differentiation of the transplanted stem cells and remaining disc cells. Hydrogels, such as collagen gel (atelocollagen), hyaluronic acid, fibrin, peptide hydrogel, polysaccharide hydrogel, and alginate, have been reported in in vivo studies to be useful as cell carriers for cell transplantation and disc regeneration therapy [49][26][50][28][51][30][32][52][53]. Degenerated discs present harsh microenvironments characterized by hypoxia, hypotrophy, acidic pH, high mechanical loading, high osmotic pressure, and a complex network of various proteases and cytokines [54][2][55][56][57][58][59]. Meanwhile, biomaterials incorporate cells into the scaffold to increase their viability, act as protective carriers to prevent the leakage of the cells from the site, and also support the transmission of mechanical loading [39]. In addition, several in vivo studies of IVD regeneration with cell-free biomaterials using hydrogels alone have reported the regenerative potential of fibrin sealant, polyglycolic acid (PGA)-hyaluronic acid scaffold, and collagen-based scaffold through hydrolysis with actinidin protease and ultra-purified alginate (UPAL) gel [22][34][60][16][61][62][63].

3. Mechanism of IVD Regeneration Therapy Using Cell-Free Soft Biomaterials Alone

Considering the various issues related to the clinical application of cell transplantation therapy, biological disc regeneration using cell-free soft biomaterials alone may be a new alternative to the current treatment for disc degeneration disease and ideally involve a single-step process [22][60]. For instance, there has been much interest in bioengineering approaches in recent years that exploit endogenous cell populations to restore the structure and function of IVDs, with the potential for IVD repair using cell-free soft biomaterials being promising [64][63]. Several in vivo studies have shown that various soft biomaterials have the potential to regenerate IVD tissue by supporting the survival and activation of remaining disc cells in damaged or degenerated IVDs and by promoting ECM production. However, details regarding their repair mechanisms have not yet been fully elucidated.

Several biomaterials have been analyzed in in vivo experiments with respect to their mechanisms in inducing and activating residual disc cells. For instance, an in vivo rabbit study of IVD aspiration followed by alginate-based hydrogel called UPAL gel implantation revealed a significant increase in the percentage of GD2Tie2 cells [22][32], which are NP progenitor cells [65]. This indicated that the implanted biomaterial was able to induce endogenous NP cells and NP progenitor cells, leading to endogenous IVD repair [22]. Similar to the UPAL gel results, a collagen type 1-based scaffold called low adhesive scaffold collagen (LASCol) promotes internal migration of the remaining disc NP cells when implanted after discectomy of rat caudal IVDs [63]. Furthermore, it has been shown that LASCol promotes the formation of cell aggregative spheroids that facilitate the maintenance of the original disc NP phenotype, upregulates the expression of chondrogenic genes, and promotes disc tissue repair [63].

Biomaterials affecting the expression of various cytokines in damaged discs have also been reported as a mechanism of biomaterial-induced disc repair. For instance, fibrin injection (fibrin sealant) after discectomy of porcine IVD has been shown to suppress acute production of proinflammatory cytokines TNF-α, IL-1β, and IL-6, increase the expression of pro-resolution cytokines IL-4 and TGF-β, and inhibit discectomy-induced progressive fibrosis of NP [61]. Furthermore, hyaluronan treatment after rat tail disc injury regulates inflammation by downregulating IFNα, reduces cell death by suppressing the expression of IGFBP3 and caspase-3 fragment p17, and induces the production of ECM [66].

4. Effects of Biomaterials on Reduction in Pain Related to Damaged IVDs

The goal of biomaterial-based IVD therapy is to not only inhibit tissue degeneration but also to control the pain caused by disc injury and degeneration. Inflammation within the lumbar IVD is often a key factor in acute low back pain [67][68][69]. Intradiscal inflammation and sensory nerve ingrowth into the deep inner layers of the AF cause discogenic pain during the chronic phase of IVD damage and degeneration [67][70]. Several types of soft biomaterials proposed as candidates for IVD repair have been shown to inhibit inflammatory cytokines in IVDs and are expected to reduce pain. Recently, it was reported in an in vivo rat IVD injury model for which methods evaluating pain-related behavior were established that hydrogels suppress pain [67][71]. Meanwhile, implantation of a hydrogel (hyaluronic acid hydrogel and UPAL gel) in a rat caudal NP punch model inhibited nociceptive behavior in Hargreaves, von Fley, and tail-flick tests [67][71]. The following possible mechanism of the hydrogel effect in the IVD injury-induced pain model has been reported. First, hydrogels implanted into injured discs of rats have been shown to regulate inflammation by inhibiting the downstream signaling cascade that activates nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) by downregulating IL-6 and IL-β and by inhibiting their binding to receptors [71]. Second, discogenic pain in the chronic phase is caused by an increased expression of nerve growth factor (NGF) that is induced by proinflammatory cytokines and the binding of NGF to its high-affinity receptor, tyrosine kinase A (TrkA), which promotes neoinnervation of the IVD and local inflammation [67][72][73][74]. Third, hydrogel treatment of injured discs has been shown to suppress neurotrophic factors, such as NGF, and

reduce NGF-TrkA binding, which mediates inhibition of neurite outgrowth of sensory nerves in the discs, resulting in reduced pain-related behavior in rats [67][71]. Finally, hydrogel treatment of damaged IVDs is expected to have a palliative effect on acute IVD pain after discectomy, as well as a preventive effect on discogenic pain [67].

5. Biomechanical Evaluation of Soft Biomaterials for NP Repair and/or Regeneration

From a mechanical perspective, soft biomaterials for use in NP treatment should ideally mimic the material properties of NP and withstand physiological loading conditions in order to restore their biomechanical properties [1]. The water content of NP is >85% by weight in juveniles, decreasing to approximately 70–75% in adults, and further decreases with additional aging and degeneration [75][76][77]. The swelling stress and effective, cohesive modulus of non-denatured human NPs in constrained compression tests are 0.138 MPa and 1.01 MPa, respectively [78], and the complex modulus of NP is 5.82 kPa at 1 rad per second, 10% compressive strain in torsional shear tests of the viscoelastic shear properties of NP [1][79]. There have been many in vitro studies on soft biomaterials that mimic the mechanical properties of native NP tissues, including alginate hydrogel, collagen gel, hyaluronic acid hydrogel, and polyethylene glycol hydrogel, among others [4][80][81][82]. It has been shown that these materials exhibit biomechanical properties comparable to those of NPs, such as water content, stiffness, and viscoelastic properties, making them candidate materials for use in NP therapy. These candidate soft biomaterials for NP treatment were first evaluated in vitro and subsequently in situ using ex vivo or in vivo preclinical animal models; however, no consensus has yet been established regarding their biomechanical evaluation as functional spinal units [1][83]. This may change as biomechanical evaluation methods have been proposed to establish best practices for screening the performance of newly developed hydrogel formulations and ensure that these materials meet minimum feasibility benchmarks for translation [83].

In general, biomechanical analysis should include (1) evaluation of the effect of hydrogel on disc function repair for axial, torsional, and viscoelastic motion segment responses and (2) evaluation of durability, mechanical feasibility, and the associated herniation risk [83]. An ex vivo approach using cadaveric animal/human motion segments can be used to investigate the biomechanical suitability of the material(s) under study [1]. Motion segments have been tested under uniaxial compression, lateral bending, and flexion/extension as a biomechanical evaluation of IVD [57]. Meanwhile, the axial compressive properties of IVDs are usually investigated in vertebra-disc-vertebra specimens of the lumbar spine, with the load-displacement curve showing a nonlinear viscoelastic response [57]. In other experiments using uniaxial compression, creep, stress relaxation, vibration/dynamic compression, and high load factor properties have been evaluated [57]. As IVDs are subjected to complex three-dimensional loading in vivo, they should be evaluated on a mechanical spine tester that can apply various combinations of cyclic compression, bending, and torsion to spinal segments ex vivo [57]. Typical moment–rotation graphs reveal marked nonlinearity and hysteresis and can be used to evaluate stiffness, the neutral zone, and range of motion. A setup using six degrees of freedom provides insight into the resulting range of motion and its restoration to previous values [1][57]. Currently, several ex vivo and in vivo studies have reported that soft biomaterials, including alginate, hyaluronic acid, chitosan-based hydrogels, and fibrin, are able to restore biomechanical disc functions, such as stiffness and range of motion, after disc implantation [22][61][84][85].

In contrast, it has been reported that hydrogel injected into IVDs may extrude out of the disc in vivo, with no improvement in biomechanical evaluation [86]. Therefore, to apply hydrogel candidates to preclinical animal models and clinical trials, it is very important to determine in situ IVD repair, a configuration for which there is currently no document to guide the evaluation of the development of new hydrogel systems for IVD treatment, including the evaluation of functional outcomes, such as implant herniation risk and structural durability [83].

Herniation risk following IVD repair has been assessed using a cyclic axial loading test and displacement-controlled ramp-to-failure test [22][87][88][83][89][90][91][92][93]. In particular, the ramp-to-failure test is designed to evaluate the worst-case IVD motion segment failure characteristics and hydrogel sealing properties as the motion segment is compressed with five degrees of side-bending and NP displacement is induced in the radial direction of the hydrogel [83]. Fatigue endurance testing has also been performed using a fatigue loading protocol established by Wilke et al. [94]. In this test, called the hula hoop test, the IVD motion segment is subjected to cyclic eccentric compression at an offset that induces a physiological bending moment until failure is reached, with NP extrusion being examined and flexibility testing being performed [88][83][94]. When assessing the risk of implant herniation and structural durability, the degree of biomechanical recovery should be assessed by comparing the motion segment of the repaired IVD with that of the intact IVD, and the biomechanical non-inferiority or superiority relative to standard treatment should be demonstrated by comparison with an IVD injury model that simulates discectomy [83].

6. Clinical Trial of Soft Biomaterials for Treating IVD Degeneration

As noted above, numerous in vitro and in vivo experiments on NP regeneration therapy based on using soft biomaterials have been performed, but very few human clinical trials have investigated the use of biomaterials alone or as a cell scaffold or delivery system for IVD regeneration. [95][2]. One of the clinical trials in which fibrin sealant was injected into the IVDs of patients with discogenic low back pain reported on the safety of the treatment with significant improvement in pain and function at a 24-month follow-up [96]. Another preliminary study showed that collagen sponges containing autologous BM-MSCs that were percutaneously transplanted into the discs of two patients resulted in improved hydration and motion segment instability of the degenerated discs and improved low back pain at two years postoperative [97]. Using a fibrin carrier in a clinical trial of 15 patients with lumbar spondylolisthesis associated with mechanical low back pain, allogeneic juvenile chondrocytes were percutaneously injected into degenerated IVDs, resulting in no apparent side effects at 12-month follow-up and significant improvement in disability and pain scores, with 77% of the patients showing improvement on MRI [23]. In a phase I study in which a combination of hyaluronic acid derivatives and autologous adipose tissue MSCs was percutaneously injected into the discs of 10 patients with chronic discogenic low back pain, there were no serious adverse events during a one-year follow-up period, and the patients showed significant improvement in visualized analog scale (VAS) and Oswestry Disability Index (ODI) scores for pain, as well as improved disc hydration on diffusion MRI [27].

References

1. Schmitz, T.C.; Salzer, E.; Crispim, J.F.; Fabra, G.T.; LeVisage, C.; Pandit, A.; Tryfonidou, M.; Maitre, C.L.; Ito, K. Characterization of biomaterials intended for use in the nucleus pulposus of degenerated intervertebral discs. Acta Biomater. 2020, 114, 1–15.
2. Huang, Y.C.; Hu, Y.; Li, Z.; Luk, K.D.K. Biomaterials for intervertebral disc regeneration: Current status and looming challenges. J. Tissue Eng. Regen. Med. 2018, 12, 2188–2202.
3. Nachemson, A.L.; Evans, J.H. Some mechanical properties of the third human lumbar interlaminar ligament (ligamentum flavum). J. Biomech 1968, 1, 211–220.
4. Pereira, D.R.; Silva-Correia, J.; Oliveira, J.M.; Reis, R.L. Hydrogels in acellular and cellular strategies for intervertebral disc regeneration. J. Tissue Eng. Regen. Med. 2013, 7, 85–98.
5. Iatridis, J.C.; Nicoll, S.B.; Michalek, A.J.; Walter, B.A.; Gupta, M.S. Role of biomechanics in intervertebral disc degeneration and regenerative therapies: What needs repairing in the disc and what are promising biomaterials for its repair? Spine J. 2013, 13, 243–262.
6. Carl, A.; Ledet, E.; Yuan, H.; Sharan, A. New developments in nucleus pulposus replacement technology. Spine J. 2004, 4, 325S–329S.
7. Coric, D.; Mummaneni, P.V. Nucleus replacement technologies. J. Neurosurg. Spine 2008, 8, 115–120.
8. Goins, M.L.; Wimberley, D.W.; Yuan, P.S.; Fitzhenry, L.N.; Vaccaro, A.R. Nucleus pulposus replacement: An emerging technology. Spine J. 2005, 5, 317S–324S.
9. Pelletier, M.H.; Cohen, C.S.; Ducheyne, P.; Walsh, W.R. Restoring Segmental Biomechanics Through Nucleus Augmentation: An In Vitro Study. Clin. Spine Surg. 2016, 29, 461–467.
10. Schutgens, E.M.; Tryfonidou, M.A.; Smit, T.H.; Oner, F.C.; Krouwels, A.; Ito, K.; Creemers, L.B. Biomaterials for intervertebral disc regeneration: Past performance and possible future strategies. Eur. Cell Mater. 2015, 30, 210–231.
11. Ruan, D.K.; Xin, H.; Zhang, C.; Wang, C.; Xu, C.; Li, C.; He, Q. Experimental intervertebral disc regeneration with tissue-engineered composite in a canine model. Tissue Eng. Part. A 2010, 16, 2381–2389.
12. Liang, C.Z.; Li, H.; Tao, Y.Q.; Peng, L.H.; Gao, J.Q.; Wu, J.J.; Li, F.C.; Hua, J.M.; Chen, Q.X. Dual release of dexamethasone and TGF-beta3 from polymeric microspheres for stem cell matrix accumulation in a rat disc degeneration model. Acta Biomater. 2013, 9, 9423–9433.
13. Buckley, C.T.; Hoyland, J.A.; Fujii, K.; Pandit, A.; Iatridis, J.C.; Grad, S. Critical aspects and challenges for intervertebral disc repair and regeneration-Harnessing advances in tissue engineering. JOR Spine 2018, 1, e1029.
14. Richardson, S.M.; Curran, J.M.; Chen, R.; Vaughan-Thomas, A.; Hunt, J.A.; Freemont, A.J.; Hoyland, J.A. The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. Biomaterials 2006, 27, 4069–4078.
15. El-Amin, S.F.; Lu, H.H.; Khan, Y.; Burems, J.; Mitchell, J.; Tuan, R.S.; Laurencin, C.T. Extracellular matrix production by human osteoblasts cultured on biodegradable polymers applicable for tissue engineering. Biomaterials 2003, 24, 1213–1221.

16. Endres, M.; Abbushi, A.; Thomale, U.W.; Cabraja, M.; Kroppenstedt, S.N.; Morawietz, L.; Casalis, P.A.; Zenclussen, M.L.; Lemke, A.J.; Horn, P.; et al. Intervertebral disc regeneration after implantation of a cell-free bioresorbable implant in a rabbit disc degeneration model. Biomaterials 2010, 31, 5836–5841.
17. Feng, G.; Jin, X.; Hu, J.; Ma, H.; Gupte, M.J.; Liu, H.; Ma, P.X. Effects of hypoxias and scaffold architecture on rabbit mesenchymal stem cell differentiation towards a nucleus pulposus-like phenotype. Biomaterials 2011, 32, 8182–8189.
18. Kim, H.Y.; Kim, H.N.; Lee, S.J.; Song, J.E.; Kwon, S.Y.; Chung, J.W.; Lee, D.; Khang, G. Effect of pore sizes of PLGA scaffolds on mechanical properties and cell behaviour for nucleus pulposus regeneration in vivo. J. Tissue Eng. Regen. Med. 2017, 11, 44–57.
19. Slaughter, B.V.; Khurshid, S.S.; Fisher, O.Z.; Khademhosseini, A.; Peppas, N.A. Hydrogels in regenerative medicine. Adv. Mater. 2009, 21, 3307–3329.
20. Yamada, K.; Sudo, H.; Iwasaki, K.; Sasaki, N.; Higashi, H.; Kameda, Y.; Ito, M.; Takahata, M.; Abumi, K.; Minami, A.; et al. Caspase 3 silencing inhibits biomechanical overload-induced intervertebral disk degeneration. Am. J. Pathol. 2014, 184, 753–764.
21. Priyadarshani, P.; Li, Y.; Yao, L. Advances in biological therapy for nucleus pulposus regeneration. Osteoarthr. Cartil. 2016, 24, 206–212.
22. Tsujimoto, T.; Sudo, H.; Todoh, M.; Yamada, K.; Iwasaki, K.; Ohnishi, T.; Hirohama, N.; Nonoyama, T.; Ukeba, D.; Ura, K.; et al. An acellular bioresorbable ultra-purified alginate gel promotes intervertebral disc repair: A preclinical proof-of-concept study. eBioMedicine 2018, 37, 521–534.
23. Coric, D.; Pettine, K.; Sumich, A.; Boltes, M.O. Prospective study of disc repair with allogeneic chondrocytes presented at the 2012 Joint Spine Section Meeting. J. Neurosurg. Spine 2013, 18, 85–95.
24. Acosta, F.L., Jr.; Metz, L.; Adkisson, H.D.; Liu, J.; Carruthers-Liebenberg, E.; Milliman, C.; Maloney, M.; Lotz, J.C. Porcine intervertebral disc repair using allogeneic juvenile articular chondrocytes or mesenchymal stem cells. Tissue Eng. Part. A 2011, 17, 3045–3055.
25. Allon, A.A.; Aurouer, N.; Yoo, B.B.; Liebenberg, E.C.; Buser, Z.; Lotz, J.C. Structured coculture of stem cells and disc cells prevent disc degeneration in a rat model. Spine J. 2010, 10, 1089–1097.
26. Crevensten, G.; Walsh, A.J.; Ananthakrishnan, D.; Page, P.; Wahba, G.M.; Lotz, J.C.; Berven, S. Intervertebral disc cell therapy for regeneration: Mesenchymal stem cell implantation in rat intervertebral discs. Ann. Biomed. Eng. 2004, 32, 430–434.
27. Kumar, H.; Ha, D.H.; Lee, E.J.; Park, J.H.; Shim, J.H.; Ahn, T.K.; Kim, K.T.; Ropper, A.E.; Sohn, S.; Kim, C.H.; et al. Safety and tolerability of intradiscal implantation of combined autologous adipose-derived mesenchymal stem cells and hyaluronic acid in patients with chronic discogenic low back pain: 1-year follow-up of a phase I study. Stem Cell Res. Ther. 2017, 8, 262.
28. Zhou, X.; Wang, J.; Fang, W.; Tao, Y.; Zhao, T.; Xia, K.; Liang, C.; Hua, J.; Li, F.; Chen, Q. Genipin cross-linked type II collagen/chondroitin sulfate composite hydrogel-like cell delivery system induces differentiation of adipose-derived stem cells and regenerates degenerated nucleus pulposus. Acta Biomater. 2018, 71, 496–509.
29. Zhang, Z.; Li, F.; Tian, H.; Guan, K.; Zhao, G.; Shan, J.; Ren, D. Differentiation of adipose-derived stem cells toward nucleus pulposus-like cells induced by hypoxia and a three-dimensional chitosan-alginate gel scaffold in vitro. Chin. Med. J. 2014, 127, 314–321.
30. Ukeba, D.; Yamada, K.; Tsujimoto, T.; Ura, K.; Nonoyama, T.; Iwasaki, N.; Sudo, H. Bone Marrow Aspirate Concentrate Combined with in Situ Forming Bioresorbable Gel Enhances Intervertebral Disc Regeneration in Rabbits. J. Bone Jt. Surg. Am. 2021, 103, e31.
31. Oehme, D.; Ghosh, P.; Shimmon, S.; Wu, J.; McDonald, C.; Troupis, J.M.; Goldschlager, T.; Rosenfeld, J.V.; Jenkin, G. Mesenchymal progenitor cells combined with pentosan polysulfate mediating disc regeneration at the time of microdiscectomy: A preliminary study in an ovine model. J. Neurosurg. Spine 2014, 20, 657–669.
32. Ukeba, D.; Sudo, H.; Tsujimoto, T.; Ura, K.; Yamada, K.; Iwasaki, N. Bone marrow mesenchymal stem cells combined with ultra-purified alginate gel as a regenerative therapeutic strategy after discectomy for degenerated intervertebral discs. eBioMedicine 2020, 53, 102698.
33. Wang, H.; Zhou, Y.; Huang, B.; Liu, L.T.; Liu, M.H.; Wang, J.; Li, C.Q.; Zhang, Z.F.; Chu, T.W.; Xiong, C.J. Utilization of stem cells in alginate for nucleus pulposus tissue engineering. Tissue Eng. Part. A 2014, 20, 908–920.
34. Leckie, S.K.; Sowa, G.A.; Bechara, B.P.; Hartman, R.A.; Coelho, J.P.; Witt, W.T.; Dong, Q.D.; Bowman, B.W.; Bell, K.M.; Vo, N.V.; et al. Injection of human umbilical tissue-derived cells into the nucleus pulposus alters the course of intervertebral disc degeneration in vivo. Spine J. 2013, 13, 263–272.

35. Mizuno, H.; Roy, A.K.; Vacanti, C.A.; Kojima, K.; Ueda, M.; Bonassar, L.J. Tissue-engineered composites of anulus fibrosus and nucleus pulposus for intervertebral disc replacement. Spine 2004, 29, 1290–1297; discussion 1297–1298.

36. Collin, E.C.; Grad, S.; Zeugolis, D.I.; Vinatier, C.S.; Clouet, J.R.; Guicheux, J.J.; Weiss, P.; Alini, M.; Pandit, A.S. An injectable vehicle for nucleus pulposus cell-based therapy. Biomaterials 2011, 32, 2862–2870.

37. Benz, K.; Stippich, C.; Fischer, L.; Mohl, K.; Weber, K.; Lang, J.; Steffen, F.; Beintner, B.; Gaissmaier, C.; Mollenhauer, J.A. Intervertebral disc cell- and hydrogel-supported and spontaneous intervertebral disc repair in nucleotomized sheep. Eur. Spine J. 2012, 21, 1758–1768.

38. Halloran, D.O.; Grad, S.; Stoddart, M.; Dockery, P.; Alini, M.; Pandit, A.S. An injectable cross-linked scaffold for nucleus pulposus regeneration. Biomaterials 2008, 29, 438–447.

39. Wei, A.; Shen, B.; Williams, L.; Diwan, A. Mesenchymal stem cells: Potential application in intervertebral disc regeneration. Transl. Pediatr. 2014, 3, 71–90.

40. Bron, J.L.; Vonk, L.A.; Smit, T.H.; Koenderink, G.H. Engineering alginate for intervertebral disc repair. J. Mech. Behav. Biomed. Mater. 2011, 4, 1196–1205.

41. Chou, A.I.; Akintoye, S.O.; Nicoll, S.B. Photo-crosslinked alginate hydrogels support enhanced matrix accumulation by nucleus pulposus cells in vivo. Osteoarthr. Cartil. 2009, 17, 1377–1384.

42. Chou, A.I.; Nicoll, S.B. Characterization of photocrosslinked alginate hydrogels for nucleus pulposus cell encapsulation. J. Biomed. Mater. Res. A 2009, 91, 187–194.

43. Guo, J.F.; Jourdian, G.W.; MacCallum, D.K. Culture and growth characteristics of chondrocytes encapsulated in alginate beads. Connect. Tissue Res. 1989, 19, 277–297.

44. Larsen, B.; Haug, A. Biosynthesis of alginate. 1. Composition and structure of alginate produced by Azotobacter vinelandii (Lipman). Carbohydr. Res. 1971, 17, 287–296.

45. Leone, G.; Torricelli, P.; Chiumiento, A.; Facchini, A.; Barbucci, R. Amidic alginate hydrogel for nucleus pulposus replacement. J. Biomed. Mater. Res. A 2008, 84, 391–401.

46. Li, Z.; Gunn, J.; Chen, M.H.; Cooper, A.; Zhang, M. On-site alginate gelation for enhanced cell proliferation and uniform distribution in porous scaffolds. J. Biomed. Mater. Res. A 2008, 86, 552–559.

47. Malafaya, P.B.; Silva, G.A.; Reis, R.L. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv. Drug Deliv. Rev. 2007, 59, 207–233.

48. Naqvi, S.M.; Buckley, C.T. Differential response of encapsulated nucleus pulposus and bone marrow stem cells in isolation and coculture in alginate and chitosan hydrogels. Tissue Eng Part. A 2015, 21, 288–299.

49. Sakai, D.; Mochida, J.; Iwashina, T.; Hiyama, A.; Omi, H.; Imai, M.; Nakai, T.; Ando, K.; Hotta, T. Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc. Biomaterials 2006, 27, 335–345.

50. Ganey, T.; Hutton, W.C.; Moseley, T.; Hedrick, M.; Meisel, H.J. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: Experiments in a canine model. Spine 2009, 34, 2297–2304.

51. Wang, F.; Nan, L.P.; Zhou, S.F.; Liu, Y.; Wang, Z.Y.; Wang, J.C.; Feng, X.M.; Zhang, L. Injectable Hydrogel Combined with Nucleus Pulposus-Derived Mesenchymal Stem Cells for the Treatment of Degenerative Intervertebral Disc in Rats. Stem Cells Int. 2019, 2019, 8496025.

52. Omlor, G.W.; Lorenz, S.; Nerlich, A.G.; Guehring, T.; Richter, W. Disc cell therapy with bone-marrow-derived autologous mesenchymal stromal cells in a large porcine disc degeneration model. Eur. Spine J. 2018, 27, 2639–2649.

53. Zhang, Y.; Drapeau, S.; Howard, S.A.; Thonar, E.J.; Anderson, D.G. Transplantation of goat bone marrow stromal cells to the degenerating intervertebral disc in a goat disc injury model. Spine 2011, 36, 372–377.

54. Huang, Y.C.; Leung, V.Y.; Lu, W.W.; Luk, K.D. The effects of microenvironment in mesenchymal stem cell-based regeneration of intervertebral disc. Spine J. 2013, 13, 352–362.

55. Gilbert, H.T.J.; Hodson, N.; Baird, P.; Richardson, S.M.; Hoyland, J.A. Acidic pH promotes intervertebral disc degeneration: Acid-sensing ion channel -3 as a potential therapeutic target. Sci. Rep. 2016, 6, 37360.

56. Huang, Y.C.; Urban, J.P.; Luk, K.D. Intervertebral disc regeneration: Do nutrients lead the way? Nat. Rev. Rheumatol. 2014, 10, 561–566.

57. Newell, N.; Little, J.P.; Christou, A.; Adams, M.A.; Adam, C.J.; Masouros, S.D. Biomechanics of the human intervertebral disc: A review of testing techniques and results. J. Mech. Behav. Biomed. Mater. 2017, 69, 420–434.

58. Risbud, M.V.; Shapiro, I.M. Role of cytokines in intervertebral disc degeneration: Pain and disc content. Nat. Rev. Rheumatol. 2014, 10, 44–56.

59. Schmidt, H.; Reitmaier, S.; Graichen, F.; Shirazi-Adl, A. Review of the fluid flow within intervertebral discs - How could in vitro measurements replicate in vivo? J. Biomech. 2016, 49, 3133–3146.

60. Abbushi, A.; Endres, M.; Cabraja, M.; Kroppenstedt, S.N.; Thomale, U.W.; Sittinger, M.; Hegewald, A.A.; Morawietz, L.; Lemke, A.J.; Bansemer, V.G.; et al. Regeneration of intervertebral disc tissue by resorbable cell-free polyglycolic acid-based implants in a rabbit model of disc degeneration. Spine 2008, 33, 1527–1532.

61. Buser, Z.; Kuelling, F.; Liu, J.; Liebenberg, E.; Thorne, K.J.; Coughlin, D.; Lotz, J.C. Biological and biomechanical effects of fibrin injection into porcine intervertebral discs. Spine 2011, 36, E1201–E1209.

62. Woiciechowsky, C.; Abbushi, A.; Zenclussen, M.L.; Casalis, P.; Kruger, J.P.; Freymann, U.; Endres, M.; Kaps, C. Regeneration of nucleus pulposus tissue in an ovine intervertebral disc degeneration model by cell-free resorbable polymer scaffolds. J. Tissue Eng. Regen. Med. 2014, 8, 811–820.

63. Takeoka, Y.; Yurube, T.; Morimoto, K.; Kunii, S.; Kanda, Y.; Tsujimoto, R.; Kawakami, Y.; Fukase, N.; Takemori, T.; Omae, K.; et al. Reduced nucleotomy-induced intervertebral disc disruption through spontaneous spheroid formation by the Low Adhesive Scaffold Collagen (LASCol). Biomaterials 2020, 235, 119781.

64. Bowles, R.D.; Setton, L.A. Biomaterials for intervertebral disc regeneration and repair. Biomaterials 2017, 129, 54–67.

65. Sakai, D.; Nakamura, Y.; Nakai, T.; Mishima, T.; Kato, S.; Grad, S.; Alini, M.; Risbud, M.V.; Chan, D.; Cheah, K.S.; et al. Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. Nat. Commun. 2012, 3, 1264.

66. Kazezian, Z.; Sakai, D.; Pandit, A. Hyaluronic Acid Microgels Modulate Inflammation and Key Matrix Molecules toward a Regenerative Signature in the Injured Annulus Fibrosus. Adv. Biosyst. 2017, 1, e1700077.

67. Ura, K.; Yamada, K.; Tsujimoto, T.; Ukeba, D.; Iwasaki, N.; Sudo, H. Ultra-purified alginate gel implantation decreases inflammatory cytokine levels, prevents intervertebral disc degeneration, and reduces acute pain after discectomy. Sci. Rep. 2021, 11, 638.

68. Miyagi, M.; Ishikawa, T.; Kamoda, H.; Suzuki, M.; Murakami, K.; Shibayama, M.; Orita, S.; Eguchi, Y.; Arai, G.; Sakuma, Y.; et al. ISSLS prize winner: Disc dynamic compression in rats produces long-lasting increases in inflammatory mediators in discs and induces long-lasting nerve injury and regeneration of the afferent fibers innervating discs: A pathomechanism for chronic discogenic low back pain. Spine 2012, 37, 1810–1818.

69. Burke, J.G.; Watson, R.W.; McCormack, D.; Dowling, F.E.; Walsh, M.G.; Fitzpatrick, J.M. Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. J. Bone Joint. Surg. Br. 2002, 84, 196–201.

70. Freemont, A.J.; Watkins, A.; Le Maitre, C.; Baird, P.; Jeziorska, M.; Knight, M.T.; Ross, E.R.; O'Brien, J.P.; Hoyland, J.A. Nerve growth factor expression and innervation of the painful intervertebral disc. J. Pathol. 2002, 197, 286–292.

71. Mohd Isa, I.L.; Abbah, S.A.; Kilcoyne, M.; Sakai, D.; Dockery, P.; Finn, D.P.; Pandit, A. Implantation of hyaluronic acid hydrogel prevents the pain phenotype in a rat model of intervertebral disc injury. Sci. Adv. 2018, 4, eaaq0597.

72. Lotz, J.C.; Ulrich, J.A. Innervation, inflammation, and hypermobility may characterize pathologic disc degeneration: Review of animal model data. J. Bone Jt. Surg. Am. 2006, 88 (Suppl. 2), 76–82.

73. Aoki, Y.; Takahashi, Y.; Ohtori, S.; Moriya, H.; Takahashi, K. Distribution and immunocytochemical characterization of dorsal root ganglion neurons innervating the lumbar intervertebral disc in rats: A review. Life Sci. 2004, 74, 2627–2642.

74. Woolf, C.J.; Allchorne, A.; Safieh-Garabedian, B.; Poole, S. Cytokines, nerve growth factor and inflammatory hyperalgesia: The contribution of tumour necrosis factor alpha. Br. J. Pharmacol. 1997, 121, 417–424.

75. Sobajima, S.; Kim, J.S.; Gilbertson, L.G.; Kang, J.D. Gene therapy for degenerative disc disease. Gene Ther. 2004, 11, 390–401.

76. Hallen, A. Hexosamine and ester sulfate content of human nucleus pulposus at different ages. Acta Chem. Scand. 1958, 12, 1869–1872.

77. Hallen, A. The collagen and ground substance of the human nucleus pulposus at different ages. Acta Chem. Scand. 1962, 16, 705–709.

78. Johannessen, W.; Elliott, D.M. Effects of degeneration on the biphasic material properties of human nucleus pulposus in confined compression. Spine 2005, 30, 724–729.

79. Iatridis, J.C.; Setton, L.A.; Weidenbaum, M.; Mow, V.C. Alterations in the mechanical behavior of the human lumbar nucleus pulposus with degeneration and aging. J. Orthop. Res. 1997, 15, 318–322.

80. Cloyd, J.M.; Malhotra, N.R.; Weng, L.; Chen, W.; Mauck, R.L.; Elliott, D.M. Material properties in unconfined compression of human nucleus pulposus, injectable hyaluronic acid-based hydrogels and tissue engineering scaffolds. Eur. Spine J. 2007, 16, 1892–1898.

81. Bron, J.L.; Koenderink, G.H.; Everts, V.; Smit, T.H. Rheological characterization of the nucleus pulposus and dense collagen scaffolds intended for functional replacement. J. Orthop. Res. 2009, 27, 620–626.
82. Vernengo, J.; Fussell, G.W.; Smith, N.G.; Lowman, A.M. Evaluation of novel injectable hydrogels for nucleus pulposus replacement. J. Biomed. Mater. Res. B. Appl. Biomater. 2008, 84, 64–69.
83. DiStefano, T.J.; Shmukler, J.O.; Danias, G.; Iatridis, J.C. The Functional Role of Interface Tissue Engineering in Annulus Fibrosus Repair: Bridging Mechanisms of Hydrogel Integration with Regenerative Outcomes. ACS Biomater. Sci. Eng. 2020, 6, 6556–6586.
84. Malhotra, N.R.; Han, W.M.; Beckstein, J.; Cloyd, J.; Chen, W.; Elliott, D.M. An injectable nucleus pulposus implant restores compressive range of motion in the ovine disc. Spine 2012, 37, E1099–E1105.
85. Gullbrand, S.E.; Schaer, T.P.; Agarwal, P.; Bendigo, J.R.; Dodge, G.R.; Chen, W.; Elliott, D.M.; Mauck, R.L.; Malhotra, N.R.; Smith, L.J. Translation of an injectable triple-interpenetrating-network hydrogel for intervertebral disc regeneration in a goat model. Acta Biomater. 2017, 60, 201–209.
86. Reitmaier, S.; Kreja, L.; Gruchenberg, K.; Kanter, B.; Silva-Correia, J.; Oliveira, J.M.; Reis, R.L.; Perugini, V.; Santin, M.; Ignatius, A.; et al. In vivo biofunctional evaluation of hydrogels for disc regeneration. Eur. Spine J. 2014, 23, 19–26.
87. Smith, L.J.; Gorth, D.J.; Showalter, B.L.; Chiaro, J.A.; Beattie, E.E.; Elliott, D.M.; Mauck, R.L.; Chen, W.; Malhotra, N.R. In vitro characterization of a stem-cell-seeded triple-interpenetrating-network hydrogel for functional regeneration of the nucleus pulposus. Tissue Eng. Part A 2014, 20, 1841–1849.
88. Lin, H.A.; Varma, D.M.; Hom, W.W.; Cruz, M.A.; Nasser, P.R.; Phelps, R.G.; Iatridis, J.C.; Nicoll, S.B. Injectable cellulose-based hydrogels as nucleus pulposus replacements: Assessment of in vitro structural stability, ex vivo herniation risk, and in vivo biocompatibility. J. Mech. Behav. Biomed. Mater. 2019, 96, 204–213.
89. Vergroesen, P.P.; Bochyn Ska, A.I.; Emanuel, K.S.; Sharifi, S.; Kingma, I.; Grijpma, D.W.; Smit, T.H. A biodegradable glue for annulus closure: Evaluation of strength and endurance. Spine 2015, 40, 622–628.
90. Cruz, M.A.; Hom, W.W.; DiStefano, T.J.; Merrill, R.; Torre, O.M.; Lin, H.A.; Hecht, A.C.; Illien-Junger, S.; Iatridis, J.C. Cell-Seeded Adhesive Biomaterial for Repair of Annulus Fibrosus Defects in Intervertebral Discs. Tissue Eng. Part. A. 2018, 24, 187–198.
91. Long, R.G.; Rotman, S.G.; Hom, W.W.; Assael, D.J.; Illien-Junger, S.; Grijpma, D.W.; Iatridis, J.C. In vitro and biomechanical screening of polyethylene glycol and poly(trimethylene carbonate) block copolymers for annulus fibrosus repair. J. Tissue Eng. Regen. Med. 2018, 12, 727–736.
92. Chik, T.K.; Ma, X.Y.; Choy, T.H.; Li, Y.Y.; Diao, H.J.; Teng, W.K.; Han, S.J.; Cheung, K.M.; Chan, B.P. Photochemically crosslinked collagen annulus plug: A potential solution solving the leakage problem of cell-based therapies for disc degeneration. Acta Biomater. 2013, 9, 8128–8139.
93. DiStefano, T.J.; Shmukler, J.O.; Danias, G.; Di Pauli von Treuheim, T.; Hom, W.W.; Goldberg, D.A.; Laudier, D.M.; Nasser, P.R.; Hecht, A.C.; Nicoll, S.B.; et al. Development of a two-part biomaterial adhesive strategy for annulus fibrosus repair and ex vivo evaluation of implant herniation risk. Biomaterials 2020, 258, 120309.
94. Wilke, H.J.; Ressel, L.; Heuer, F.; Graf, N.; Rath, S. Can prevention of a reherniation be investigated? Establishment of a herniation model and experiments with an anular closure device. Spine 2013, 38, 587–593.
95. Binch, A.L.A.; Fitzgerald, J.C.; Growney, E.A.; Barry, F. Cell-based strategies for IVD repair: Clinical progress and translational obstacles. Nat. Rev. Rheumatol. 2021, 17, 158–175.
96. Yin, W.; Pauza, K.; Olan, W.J.; Doerzbacher, J.F.; Thorne, K.J. Intradiscal injection of fibrin sealant for the treatment of symptomatic lumbar internal disc disruption: Results of a prospective multicenter pilot study with 24-month follow-up. Pain Med. 2014, 15, 16–31.
97. Yoshikawa, T.; Ueda, Y.; Miyazaki, K.; Koizumi, M.; Takakura, Y. Disc regeneration therapy using marrow mesenchymal cell transplantation: A report of two case studies. Spine 2010, 35, 475–480.